Amendments to the Specification:

Please amend the substitute specification as follows:

Substitute the following paragraph for the paragraph bridging pages 1 and 2:

Since the DNA shuffling technique developed by Stemmer was published in Science in 1994, many molecular breeding technologies relating to the artificial evolution of genes have been developed (Juha P. Int Arch Allergy Immunology. 121, 173-182 (2000)), including various improved protocols of gene shuffling (Huimin Z. et al. Nucleic Acids Research, 25 (6), 1307-1 308 (1997); Andreas C. et al. Nature, 391 (15), 288-291 (1998); Miho K. et al. Gene, 236, 159-167 (1997)), staggered extension process (Huimin 7. et al. Nature Biotechnology, 16, 258-261 (1998)), incremental truncation for the creation of hybrid enzymes (Marc 0. et al. Nature Biotechnology, 17, 1205-1209 (1999)) and random chimeragenesis on transient templates (Wayne MC. et al. Nature Biotechnology, 19, 354-359 (2001)) etc. To date there are many successful examples in which the basic <u>principles</u> [pricinples] of the molecular evolution techniques have been applied to generate or modify genes in fields ranging from the common biological proteases to improvement of antibiotic titre, the degradation of pollutants in the environment, the reconstruction of viruses, and the development of pharmaceuticals. But it is rarely employed in field of DNA vaccines which is the third generation of human vaccines. Although many experts predict that the success of gene shuffling technology in gene vaccines will make it widely applicable to diseases such as cancer, autoimmune diseases and infectious diseases which severely harm human health (Dewey D.Y.R et al. Biotechnology Progress, 16 (1), 2-16 (2000); Phillip A P. et al. Current Opinion in Biotechnology, 8, 724-733 (1997); Robert G.W. et al. Curr Opin Mol Ther, 3 (1), 31-36 (2001)), there is no related literature or patents demonstrating substantial progress.

Substitute the following paragraph for the full paragraph on page 2:

Gene vaccines represent a new immunological theory and technique developed in the 1990s and are the third generation of vaccines after attenuated virus vaccines and subunit vaccines (Wolff J. A. et al. Science, 247, 1465-8 (1990)). The technology of gene

vaccines comprises the step of direct injection of plasmid DNA containing exogenous protein coding sequences into the body so as to enable the direct expression of the <u>exogenous</u> [exogeous] proteins in the body thereby eliciting an immune response. Gene vaccines have many advantages compared to conventional vaccines, such as prolonged immune response, simultaneous induction of humoral immunity and cytotoxic T cell response, simple preparation, convenience, inexpensive, stable antigen and convenient delivery, and so on. It not only has the safety proved by recombinant subunit vaccines and the high efficiency of attenuated virus vaccines for the induction of a general immune response but also elicits specific types of immune response in the body. Up to now, gene vaccines have been widely used for therapy of infectious <u>diseases</u> [diseases] and cancer caused by viruses, bacteria and protozoa as well as in the therapy of allergic response and tolerance in new born infants. There is beneficial development in the therapy against influenza, AIDS, rabies, hepatitis B, tuberculosis, malaria and leishmaniosis (Lai W.C. et al. Crit Rev Immunol, 18 (5), 449-84 (1998)). With respect to plasmodium, HIV and other highly variable viruses severely affecting human health, there are no very effective vaccines.

Please substitute the following paragraph for the paragraph bridging pages 3 and 4:

The life cycle of *Plasmodium falciparum* which causes malignant malaria severely affecting human health is complicated and comprises four stages comprising asexual reproduction and sexual reproduction in humans and sexual reproduction and sporogony in <u>mosquitoes</u> [mosquitos]. In humans there are exoerythrocytic (liver) and erythrocytic stages, while gametocyte and sporozoite stages are in <u>mosquitoes</u> [mosquitos]. Such complex biological traits cause *Plasmodium falciparum* to have highly variable response against the <u>immunoprotection</u> [immunoprotectiion] of the host and drugs, so that single protective antigenic vaccines against malaria are not effective.

Please substitute the following paragraph for the first full paragraph on page 4:

The clinical symptoms caused by plasmodium are mainly due to its asexual reproduction

in the red blood cells of the host. Erythrocytic stage vaccines are designed to act directly against this unique pathogenic stage of plasmodium. Malaria vaccines comprise attenuated circumsporozoite vaccine, subunit vaccine and synthetic peptide vaccine, but they are not successful because the various antigens against which various vaccines are directed can not generate satisfactory protective effects. Therefore, it is well accepted in the art that the combination of multi-stage and multivalent epitopes is necessary in the <u>construction</u> [construction] of a malaria vaccine, to make it possible to obtain the desired protective effect. However, it is difficult to determine the quantity and linking order of the genes encoding polypeptides during the construction of multi-stage and multivalent vaccines manually, and the induction of humoral immunity by epitope DNA vaccines is generally not satisfactory, which are problems to be solved.

Please insert the following paragraph on page 5 before the paragraph bridging pages 5 and 6:

In another aspect, the present invention provides a method for preparing polyepitope chimeric gene vaccines, comprising the steps of:

a) selecting, synthesizing and cloning Into a vector a plurality of nucleic acid molecules each encoding a single epitope of an antigen of interest;

b) constructing nucleic acid molecules encoding randomly combined bi-epitopes in the vectors of step a) by isocaudamer linkage;

c) randomly assembling polyepitope chimeric genes with different lengths from the nucleic acid molecules encoding bi-epitopes of step b);

d) selecting polyepitope chimeric genes according to different length ranges, cloning the polyepitope chimeric genes into expression vectors to obtain polyepitope chimeric gene expression libraries in the corresponding length ranges;

e) detecting differences of polyepitope chimeric genes in the polyepitope chimeric gene expression libraries to ensure the high diversity of the gene libraries used for vaccines.

Please substitute the following paragraph for the first full paragraph on page 8:

Five gene libraries were used to immunize Balb/c mice twice at 100μg DNA to generate antisera, which were 2X diluted. The negative control was used as a reference, and the positive has a fluorescence [fluorescence] intensity significantly different from that of the control.

Please substitute the following paragraph for the fifth full paragraph on page 9:

According to the method of the invention, the antigen of interest is any antigen related to various infectious diseases, tumors or autoimmune diseases. Many sequences of the antigen epitopes are known in the art, and based on these sequences it is possible to synthesize a plurality of the nucleic acid molecules, [molecule] each nucleic acid encoding a single epitope of an antigen of interest in step a).

Please substitute the following paragraph for the first full paragraph on page 10:

The obtained randomly assembled polyepitope chimeric genes with different lengths are separated according to the various length ranges which may be set according to the requirements [requirements] and which are usually from hundreds to thousands [thusands] of base pairs. In one embodiment of the invention, five groups of randomly assembled polyepitope chimeric genes of respectively 300, 800, 1200, 2000 and 4000bp are separated. It is understood that one skilled in the art may set any desired length ranges. Then these separated groups of polyepitope chimeric genes may be cloned into any appropriate expression vector known in the art after being purified and amplified, and used to transform appropriate host cells to obtain several expression libraries of polyepitope chimeric genes.

Please substitute the following paragraph for the first full paragraph on page 13:

b) A structure of ~~GLy-Pro-Gly-Pro~~ Gly-Pro-Gly-Pro (G-P-G-P) (SEQ ID NO: 1) was introduced near the *BcI*I and *Bam*HI linkage site of the different linked antigen epitopes in order to increase the steric flexibility of the epitope linkages.

Please substitute the following paragraph for the fifth full paragraph on page 13:

b) The amplification products were precipitated by 1/10 volume of 1OM ammonium acetate and 2 volume of pure ethanol [enthanol], then resolved in ultra-purified water, digested with *Bcl*I and *Bam*HI and treated with equal volume of phenol, followed by centrifugation at 12000rpm for 5 mm. The supernatant [supertanant] was precipitated by 1/10 volume of 3M sodium acetate and 2 volume of pure *ethanol* [enthanol], and then resolved in ultra purified water.

Please substitute the following paragraph for the first full paragraph on page 15:

The bi-epitope recombinant plasmids in above step 1 were mixed, subsequently cleaved with Bc/I and BamHI, and subjected to electrophoresis on a low melting point agarose [agrose] gel. The small fragments were retrieved with DNA purification kits (Promega), and measured at $OD_{260}/0D_{280}$ for their concentrations.

Please substitute the following paragraph for the paragraph bridging pages 15 and 16:

The reaction conditions (25, 35, 45, 55, 65, 75, or 85 cycles) were as follows. First procedure, 94℃, 3min; 94℃, 30sec; 42-55℃, 30sec; 72℃, 30sec; 25 cycles; and 72℃, 10min. Second procedure: 94℃, 3min; 94℃, 45sec; 50-55℃, 45sec; 72℃, 30sec, 1sec/cycle; 10 cycles; and 72℃, 10min. The products of the primer-free amplification were subjected to 1% agarose [agrose] gel electrophoresis and the results were shown in Fig 2, which showed that the assembled polyepitope gene fragments had increased length with the increase of the cycle number. Five DNA fragments with different lengths (about 300, 800, 1200, 2000 and 4000bp, respectively) were retrieved from the gel and subjected to a conventional PCR reaction with upstream primer 5'-ACATCATGCCTGATCA-3' and downstream primer5'-TTAGCTAGCGGATCC-3'. The reaction system was the same as that for the primer-free PCR, with a procedure: 94℃, 3mm; 94℃, 3Osec; 50℃, 30sec; 72℃, 30sec; 30 cycles; and 72℃ 10min. The amplification products were purified and concentrated by wizard PCR prep purification kit

(Promega), then cleaved with *BclI* and ligated into vector VR1O12 which had been cleaved with EcoRV and *BclI,* and the ligation mixture were electrotransformed into E. coli strain SK383. The methods and results were set forth in Fig. 1 and Fig. 2.

Please substitute the following paragraph for the fourth full paragraph on page 16:

Primers (1A: 5'-GATCACCATGGAATTCG-3' (SEQ. ID. No. 16) and 1B: 5'-GATCCGAATTCCATGGT-3' (SEQ ID No. 17)) containing eukaryotic Kozak sequence were designed and then were allowed to self anneal and extend. The PCR product was cleaved with *BclI* and *Bam*HI and cloned into vector VR1012 with E coli strain SK383 as the recipient strain, thereby a recombinant plasmid VR10A was obtained and sequenced by Takara Biotech Company.

Please substitute the following paragraph for the paragraph bridging pages 17 and 18:

Libraries No.3 and No.4 were tested for the single-strand conformational pleiomorphism [comformation polymorphism] (PCR-SSCP). The results indicated that the diversity in the chimeric genes of both libraries was higher than 95% (24/25 and 25/25, respectively), as shown in Fig. 5. The steps for this test were as follows:

Please substitute the following paragraph for the third full paragraph on page 18:

c) The glass with gel was put into a Petri [Petri] dish and fixing buffer (10% glacial acetic acid v/v) was added to submerge [immerge] the gel for at least 20 mm with horizontally shaking slowly. After fixation, the fixing buffer was collected and used as fixer after development.

Please substitute the following paragraph for the sixth full paragraph on page 19:

Bacteria containing the polyepitope gene libraries with different lengths (No.1, No.2, No.3, No.4 and No.5) constructed in step 2 of Example 3 were washed respectively from

the plates, and cultured in LB liquid medium with corresponding antibiotics. Then an [a] inoculum was used to inoculate 1 liter of LB liquid medium with corresponding antibiotics at a ratio of 1:100, and the bacteria were cultured to logarithmic growth phase. The bacteria [bateria] were collected. Plasmids were extracted according to Wizard Megapreps plasmid extraction kit (Promega). DNA concentration was calculated from OD_{250}/OD_{280} measured with DU7O ultraviolet spectrophotomer [spectrophotometer] (Beckman).

Please substitute the following paragraph for the paragraph bridging pages 19 and 20:

100μg DNAs of the recombinant plasmids of the gene vaccine libraries 30 obtained in above step 1 were adjusted to equal volume [volume] with sterile saline. Groups of Balb/c mice were immunized by injecting equal volume of such DNAs into humerus quadriceps of both legs, with three animals in each group. The empty vector and blank without DNA were taken as controls. Immunization was boosted every two weeks, totally three times. Blood samples were taken from each group and the polyclonal antisera were collected after immunization.

Please substitute the following paragraphs for the second and third full paragraphs on page 20:

In the presence of positive control, negative control and blank control, mixed epitope synthetic peptides were used as coating antigens. The antisera to be tested were two-fold diluted (such as 400, 800, 1600, 3200, 6400, 12800, 25600 and 51200). The maximal dilution (titre) of the antisera after immunization [immunization] with different polyepitope gene libraries were detected by ELISA, as shown in Fig. 6. The results indicated that the gene libraries with different lengths had different immunogenicity and high titers of antibodies were generated. The specific steps for the assay were as follows:

a) Coating: synthetic peptides of a single or mixed epitopes were used as coating

antigens, and diluted with a coating buffer of 0.1 M carbonate, pH 9.2 into a desired concentration (200ng/l00µl/well). 100µl was added into each well by a pipette [pipet] and then the plate was placed in humidified environment overnight at 4°C or for 4h at 37°C. The plate was decanted and rinsed with PBST five times.

Please substitute the following paragraphs for the first and second full paragraphs on page 22:

4. Detection results of indirect immunofluorescence [immunoflurescence] assay (IFA) for the recognition of native proteins by the polyclonal antisera generated [gererated] by the different polyepitope gene libraries.

In order to determine whether or not the antisera generated by the different polyepitope gene libraries recognize native proteins of *Plasmodium falciparum* and *Plasmodium yoelii,* an indirect immunofluorescence [immunoflurescence] assay (IEA) was used. In the presence of a positive control, the maximum dilution of antibody which permits the recognition [recoginition] was determined and confocal microscopy was used to determine the binding sites of antibody, the results were shown in Figs. 7 and 8. The specific steps of this assay were as follows [follow]:

Please substitute the following paragraph for the second full paragraph on page 25:

In order to construct polyepitope chimeric gene vaccines against malignant malaria more efficiently [efficiently], we tested the protection role of the gene vaccines of the invention in a P. yoelli animal model. The result indicated that the five polyepitope gene libraries all exhibited protection with varying extent (Fig. 10), which laid a foundation for an *in vivo* protection model related to the protection role of the artificial shuffled gene vaccines against *Plasrnodium falciparum.* The specific steps of the experiment were as follow:

Please substitute the following paragraph for the paragraph on page 27:

The obtained positive (high immunogenic) gene clones SP3l2. SP352 and SP462 were confirmed by *in vivo* immunization. In the presence of negative (low <u>immunogenic</u> [immunogenic]) gene clones and empty vector, Balb/c mice were immunized three times, and spleen lymphocytes were isolated for the s detection of cytokines CD4 and CD8 using flow cytometry. The results indicated that positive (high immunogenic) gene clones SP312, SP352 and SP462 predominantly induced the production of cytokine CD4, accompanied with certain level of cytokine CD8, wherein positive polyepitope gene 5P312 generated a level of cytokines markedly higher than that of 5P352 or SP462. In contrast, negative polyepitope genes behaved similar to empty vector, demonstrating that polyepitope chimeric gene vaccines with higher immunogenicity can be obtained by high-throughput immunochemistry method (Fig 12).

Please substitute the tables on the following three pages for Table 1 on page 29 and Table 2 on pages 30-31:

Table 1. Amino acid sequences of B- and Th- cell epitopes of antigens of *Plasmodium falciparum* in different life stages used in the invention

Epitope gene	Amino acids sequence	Seq. ID	Antigen gene	Life stage	Types of immunocyte [immnunocyte]	Reference
$E2_{(NKND)}$	NKNDNKND	Seq ID No:2	NKND	Cross	B	Cheng Q, 1991
$E3_{(MSA-2)}$	KNESKYSNTFINNAYNMSIRRS M	Seq ID No:3	MSA-2	Erythrocytic stage	B/Th	Symthe JA, 1991
$E4_{(RESA)}$	EENVEHDA	Seq ID No:4	RESA	Erythrocytic stage	B	Chauhan VS, 1993
$E5_{(EBA-175)}$	EREDERTLTKEYEDIVLK	Seq ID No:5	EBA-175	Erythrocytic stage	B	Sim BK, 1994; 1998
$E6_{(MSA-1)}$	LDNIKDNVGKMEDYIKKNKK	Seq ID No:6	MSA-1	Erythrocytic stage /liver stage	B/Th	Kumar A, 1992; Chauhun YS, 1993
$E7_{(LSA-1)}$	EQQSDLEQERL(R)AKEKLQ	Seq ID No:7	LSA-1	liver stage	B/Th	Aidoo M, 2000
$E8_{(CS.T3/CSP)}$	KKIAKMEKASSVFNV	Seq ID No:8	CS.T3/CSP	sporozoite stage	Tb	Sinigaglia F, 1988
$E9_{(MSP-1)}$	NSGCFRHLDEREECKCLL	Seq ID No:9	MSP-1	Erythrocytic stage	B	Chang SP, 1992
$E10_{(MSP-1)}$	EDSGSNGKKITCECTKPDS	Seq ID No:10	MSP-1	Erythrocytic stage	B	Chang SP, 1992
$E11_{(AMA-1)}$	DGNCEDIPIIVNEFSAIDL	Seq ID No:11	AMA-1	Erythrocytic stage	B	Shi YP, 1999
$E12_{(AMA-1)}$	GNAEKYDKMDEPQHYGKS	Seq ID No:12	AMA-1	Erythrocytic stage	B	Lal AA, 1996
$E15_{(AMA-1)}$	DQPKQYEQHLTDYEKIKEG	Seq ID No:13	AMA-1	Erythrocytic stage	Th	Lal AA, 1996
$E16_{(MSP-1)}$	GISYYEKVLAKYKDDLE	Seq ID No:14	MSP-1	Erythrocytic stage	Th	Udhayakumar V, 1995
$E17_{(MAg-1)}$	QTDEIKNDNI	Seq ID No:15	MAg-1	Erythrocytic stage	B/Th	Lu Y, unpublished

Table 2. Primer sequences [sequences] for cloning epitope genes

Epitope genes	Seq ID	Primer sequence
E2$_{(NKND)}$ A	Seq ID No:18	2A: 5' -ACATCATGCCT'CATCA**AACAAGAACGACAACA**-3' *Bcl*I
B	Seq ID No:19	2B: 3' -**TGTTCTTGCTGTTGTTCTTGCTG**CCTAG' GCGC-5' *Bam*HI
E3$_{(MSA-2)}$	Seq ID No:20	3A: 5' -ACATCATGCCT' GATCA**AAGAACGAGAGCAAGTACAG**- 3' *Bcl*I
A C	Seq ID No:21	3B: 3' -**GCTCTCGTTCATGTCGTTGTGGAAGTAGTTGTTGCGGATG-** 5'
	Seq ID No:22	3C: 5' -**CATCAACAACGCCTACAACATGAGCATCCGCCGCAGCATGG**- 3'
B D	Seq ID No:23	3D: 3' -**GGCGGCGTCGTAC**CCGGGGCCGGGGCCTAG'GCGC-5' *Bam*HI
E4$_{(RESA)}$ A	Seq. ID No:24	4A: 5' -ACATCATGCCT'GATCA**GAGGAGAACGTGGAGC**-3' *Bcl*I
B	Seq ID No:25	4B: 3' -**TCCTCTTGCACCTCGTGCTGCGG**CCTAG' GCGC-5' *Bam*HI
E5$_{(EBA-175)}$ A	Seq ID No:26	5A: 5' –ACATCATGCCT'GATCA**GAGCGCGAGGACGAGCGCACCCTGACCAAGGAGTACG**- 3' *Bcl*I
B	Seq ID No:27	53: 5B' -**GACTGGTTCCTCATGCTCCTGTAGCACGACTTC**CCGGGGCCGGGGCCTAG' GCGC 5' *Bam*HI
E6$_{(MSA-I)}$	Seq ID No:28	6A: 5' - ACATCATGCCT' GATCA**CTGGACAACATCAAGGACAACGTGGGC** -3' *Bcl*I
A C	Seq ID No:29	6B: 3' -**TCCTGTTGCACCCGTTCTACCTCCTGATGTAGTTCTTCTTGT**-5'
	Seq ID No:30	6C: 5' -**TACATCAAGAAGAACAAGA.AG**GGCCCCGGCC'CCGGATCCGC- 3'
B D	Seq ID No:31	6D:3' - CGGGGCCTAG' GCGATCGATTATTTCTAGAAGG- 5' *Bam*HI
E6$_{(MSA-1)}$ A	Seq ID No:32	7A: 5' -ACATCATCGCT' GATCAGA**GCAGCAGAGCGACCTGGAGCAGGAGCGCCTGG**- 3' *Bcl*I
B	Seq ID No:33	7B: 3' -**GTCCTCGCGGACCGGTTCCTCTTCGACGTC**CCGGGGCCGGGGCCTAG' GCGC- 5' *Bam*HI

$E8_{(CS.T3/CSP)}$ A	Seq ID No: 34	8A: 5' - ACATCATGCCT' GATCAAAGAAGATCGCCAAGATGGAGAAGGCCAGCAGC -3' *BclI*
B	Seq ID No: 35	8B:3' - **CTCTTCCGGTCGTCGCACAAGTTGCAC**CCGGGGCCGGGGCCTAG' GCGC- 5' *Bam*HI
$E9_{(MSP-1)}$ A	Seq ID No: 36	9A: 5' -ACATCATGCCT' GATCA**AACAGCGGCTGCTTCCGCCACCTGGACGAGCCGC**-3 *BclI*
B	Seq ID No: 37	9B: 3' -**TGGACCTGCTCGCGCTCCTCACGTTCACGGACGAC**CCTAG' GCGC~ 5' *Bam*HI
$E10_{(MSP-1)}$ A	Seq ID No: 38	10A: 5' -ACATCATGCCT' GATCA**GAGGACAGCGGCAGCAACGGCAAGAAGATCACCTGC-3'** *BclI*
B	Seq ID No: 39	10B: 3' -**GTTCTTCTAGTGGACGCTCACGTGGTTCGGCCTGTCG**CCTAG'GCGC 5' *Bam*HI
$E11_{(AMA-1)}$ A	Seq ID No: 40	11A: 5'-ACATCATGCCT' GATCA**GACGGCAACTGCGAGGACATCCCGCACGTGAAC-3'** *BclI*
B	Seq ID No: 41	11B:3'-**TAGGGCGTGCACTTGCTCAAGTCGCGGTAGCTGGAC**CCTAG'GCGC- 5 *Bam*HI
$E12_{(AMA-1)}$ A	Seq ID No: 42	12A: 5' -ACATCATGCCT' GATCA**GGCAACGCCGAGAAGTACGACAAGATGGACGAGCCG-3'** *BclI*
B	Seq ID No: 43	12B: 3' -**TTCTACCTGCTCGGCGTCGTGATGCCGTTCTCG**CCTAG'GCGC- 5' *Bam*HI
$E15_{(AMA-1)}$ A	Seq ID No: 44	15A: 5' -ACATCATGCCT'GATCA**GACCAGCCGAAGCAGTACGAGCAGCACCTGACCGAC- 3'** *BclI*
B	Seq ID No: 45	15B: 3' -**GTCGTGGACTGGCTGATGCTCTTCTAGTTCCTCCCG**CCTAG'GCGC- 5' *Bam*HI
$E16_{(MSP-1)}$ A	Seq ID No: 46	16A: 5' -ACATCATGCCT'GATC**AGGCATCAGCTACTACGAGAAGGTGCTGGCCAAG-3'** *BclI*
B		~~16B: 3' -GTCGTGGACTGGCTGATGCTCTTCTAGTTCCTCCCGCCTAG'GCGC-5' BamHI~~
B	Seq ID No: 47	16B: 3' –**TTCCACGACCGGTTCATGTTCCTGCTGGACCTC**CCTAG'GCGC - 5' *Bam*HI
$E17_{(MAg-1)}$ A	Seq ID No: 48	17A:5' – ACATCATGCCT'GATCA**CAGACCGACGAGATCAAGAACGACCACATCCAGACCGAT-3'** *BclI*
B	Seq ID No: 49	17B: 3' -**GTGTAGGTCTGGCTACTTTAATTTTTACTATTATAA**CCTAG'GCGC- 5 *Bam*HI